

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 35/37, 35/38	A1	(11) International Publication Number: WO 98/10775 (43) International Publication Date: 19 March 1998 (19.03.98)
(21) International Application Number: PCT/US97/16294 (22) International Filing Date: 16 September 1997 (16.09.97) (30) Priority Data: 60/026,197 16 September 1996 (16.09.96) US (71) Applicant (for all designated States except US): PURDUE RESEARCH FOUNDATION [US/US]; Room 307, 1021 Hovde Hall, West Lafayette, IN 47907-1021 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): COBB, Mark. A. [US/US]; 3200 Oakridge Court, Newburgh, IN 47630 (US). BADYLAK, Stephen, F. [US/US]; 2610 Nottingham Place, West Lafayette, IN 47906 (US). ISOM, Gary [US/US]; 30 Brynteg Estates, West Lafayette, IN 47906 (US). SHARMA, Archana [IN/US]; 2127 Old Oak Drive, West Lafayette, IN 47906 (US). (74) Agents: LAMMERT, Steven, R. et al.; Barnes & Thornburg, 11 South Meridian Street, Indianapolis, IN 46204 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: COMPOSITION AND METHOD FOR REPAIRING NEUROLOGICAL TISSUE (57) Abstract The present invention relates to tissue graft constructs useful in promoting regrowth and healing of damaged or diseased neurological related tissue structures. More particularly this invention is directed to a submucosa tissue graft construct and a method of inducing the formation of endogenous neurological structures at a site in need of endogenous neurological related tissue growth.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NI	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

- 1 -

COMPOSITION AND METHOD FOR REPAIRING NEUROLOGICAL TISSUE5 Field of the Invention

 This invention relates to tissue graft constructs useful in promoting regrowth and healing of damaged or diseased neurological related tissue structures. More particularly this invention is directed to a method of inducing the formation of endogenous neurological structures at a site in need of endogenous neurological related
10 tissue growth by contacting the site with a submucosal tissue graft construct.

Background and Summary of the Invention

 The neurosurgeon is frequently confronted with the necessity of repairing dural defects due to trauma, tumor resection, and decompressive procedures. Numerous
15 materials have been investigated for use in the repair of the dura mater and underlying tissues. Current options include autologous materials (e.g. pericranium, temporalis fascia, and tensor fascia lata), lyophilized cadaveric materials (e.g. dura mater and tensor fascia lata) and synthetic materials (e.g. Silastic sheets, Dacron sheets, Vicryl mesh); however, each of these materials is associated with significant limitations.

20 One object of the present invention is to provide a biodegradable material that can serve as a dural substitute.

 Many individuals have suffered injuries to their central nervous system that leave the individual partially paralyzed or result in reduced motor function. Repair strategies and graft material for repairing damage to the central nervous system do not
25 currently exist. In particular nerve fibers within the brain and the spinal cord, which differ structurally from peripheral nerves, will not regenerate after they have been severed or crushed. For example there is no currently known treatment for humans that promotes functional regeneration across a complete spinal cord transection or a severed optic nerve.

 An additional object of the present invention is to provide a composition
30 and method that promotes the production of endogenous central nerve cells thus allowing the repair of damage to both central nervous system tissues and peripheral nerve tissues.

- 2 -

It is known that compositions comprising the tunica submucosa delaminated from both the tunica muscularis and at least the luminal portion of the tunica mucosa of the intestine of warm-blooded vertebrates can be used as tissue graft materials. See, for example, U.S. Patent Nos. 4,902,508 and 5,281,422. The compositions described in those patents are characterized by excellent mechanical properties, including high compliance, a high burst pressure point, and an effective porosity index which allowed such compositions to be used beneficially for vascular graft constructs and in connective tissue replacement applications. When used in such applications the submucosal graft constructs appear to serve as a matrix for the regrowth of the tissues replaced by the graft constructs. Furthermore, as described in U.S. Patent No. 5,275,826 fluidized forms of vertebrate submucosal tissues can also be used as injectable or implantable tissue grafts without loss of biotropic properties. Significantly, too, in over 600 cross-species implants, submucosa-derived graft compositions have never been shown to elicitate a tissue graft rejection reaction.

Applicants have discovered that submucosal tissue induces the growth and proliferation of neurological related tissues, including the dura mater and nerve cells of the central and peripheral nervous system. Accordingly, the present invention is directed to the use of submucosal tissue as a graft construct for promoting the repair of damaged or diseased neurological related tissues.

20

Detailed Description of the Preferred Embodiments

The submucosal tissue constructs of the present invention have been found to promote or induce the growth of neurological related tissues. In accordance with the present invention the term neurological related tissues includes neurons and glial cells, and dura mater, arachnoid and pia mater tissues. There is provided in accordance with this invention a method for utilizing compositions comprising warm-blooded vertebrate submucosal tissue to repair or to enhance the repair of damaged or diseased neurological related tissues in a warm-blooded vertebrate. The method comprises the step of contacting the site in need of repair with a composition comprising submucosal tissue.

- 3 -

Submucosal tissue suitable for use in accordance with the present invention comprises natural collagenous matrices that include highly conserved collagens, glycoproteins, proteoglycans, and glycosaminoglycans in their natural configuration and natural concentration. One source of submucosal tissue is the intestinal tissue of a warm-blooded vertebrate. Small intestinal tissue is a preferred source of submucosal tissue for use in this invention.

Submucosal tissue for use in this invention is derived from various warm-blooded vertebrate sources, including intestinal tissue harvested from animals raised for meat production, such as pigs, cattle and sheep or other warm-blooded vertebrates. This tissue can be used in either its natural configuration or in a comminuted or partially enzymatically digested fluidized form. Vertebrate submucosal tissue is a plentiful by-product of commercial meat production operations and is thus a low cost graft material, especially when the submucosal tissue is used in its native layer sheet configuration.

Suitable intestinal submucosal tissue typically comprises the tunica submucosa delaminated from both the tunica muscularis and at least the luminal portion of the tunica mucosa. In one embodiment of the present invention the intestinal submucosal tissue comprises the tunica submucosa and basilar portions of the tunica mucosa including the lamina muscularis mucosa and the stratum compactum. Those layers are known to vary in thickness and in definition dependent on the source vertebrate species.

The preparation of submucosal tissue for use in accordance with this invention is described in U.S. Patent No. 4,902,508, the disclosure of which is expressly incorporated herein by reference. A segment of vertebrate intestine, preferably harvested from porcine, ovine or bovine species, but not excluding other species, is subjected to abrasion using a longitudinal wiping motion to remove the outer layers, comprising smooth muscle tissues, and the innermost layer, *i.e.*, the luminal portion of the tunica mucosa. The submucosal tissue is rinsed with saline and optionally sterilized; it can be stored in a hydrated or dehydrated state. Lyophilized or air dried submucosa tissue can be rehydrated and used in accordance with this invention without significant loss of its biotrophic and mechanical properties.

- 4 -

Submucosal tissue prepared from warm-blooded vertebrate organs typically has an abluminal and a luminal surface. The luminal surface is the submucosal surface facing the lumen of the organ source and typically adjacent to an inner mucosa layer in the organ source, whereas the abluminal surface is the submucosal surface facing
5 away from the lumen of the organ source and typically in contact with smooth muscle tissue in the organ source.

The submucosal tissue graft compositions of the present invention can be preconditioned by stretching the material in a longitudinal or lateral direction as described in U.S. Patent No. 5,275,826, the disclosure of which is expressly incorporated herein by
10 reference. Multiple strips/pieces of submucosal tissue can also be fused together to form a unitary multi-layered submucosal tissue construct having a surface area greater than any to the individual strips/pieces of submucosal tissue. The process for forming large area/multilayered submucosal tissue constructs is described in U.S. Patent Application 08/418,515, the disclosure of which is expressly incorporated herein by reference. In
15 summary, the process of forming large area sheets of submucosal tissue comprises overlapping at least a portion of one strip of submucosal tissue with at least a portion of another strip of submucosal tissue and applying pressure at least to said overlapped portions under conditions allowing dehydration of the submucosal tissue. Under these conditions the overlapped portions will become "fused" to form a unitary large sheet of
20 tissue.

The large area graft constructs consist essentially of submucosal tissue, free of potentially compromising adhesives and chemical pretreatments, and they have a greater surface area and greater mechanical strength than the individual strips used to form the graft construct. The multilayered submucosal constructs can optionally be
25 perforated as described in U.S. Patent Application 08/418,515, the disclosure of which is expressly incorporated herein by reference. The perforations of the submucosal tissue construct allow extracellular fluids to pass through the tissue graft material, decreasing fluid retention within the graft and enhancing the remodeling properties of the tissue grafts. The perforation of the submucosal tissue is especially beneficial for multi-laminate

- 5 -

tissue graft constructs wherein the perforations also enhance the adhesive force between adjacent layers.

The submucosal tissue specified for use in accordance with this invention can also be in a fluidized form. Submucosal tissue can be fluidized by comminuting the tissue and optionally subjecting it to enzymatic digestion to form a substantially homogenous solution. The preparation of fluidized forms of submucosa tissue is described in U.S. Patent No. 5,275,826, the disclosure of which is expressly incorporated herein by reference. Fluidized forms of submucosal tissue are prepared by comminuting submucosa tissue by tearing, cutting, grinding, or shearing the harvested submucosal tissue. Thus pieces of submucosal tissue can be comminuted by shearing in a high speed blender, or by grinding the submucosa in a frozen or freeze-dried state to produce a powder that can thereafter be hydrated with water or a buffered saline solution to form a submucosal fluid of liquid, gel-like or paste-like consistency. The fluidized submucosa formulation can further be treated with enzymes such as protease, including trypsin or pepsin at an acidic pH, for a period of time sufficient to solubilize all or a major portion of the submucosal tissue components and optionally filtered to provide a homogenous solution of partially solubilized submucosa.

The graft compositions of the present invention can be sterilized using conventional disinfection/sterilization techniques including glutaraldehyde tanning, formaldehyde tanning at acidic pH, propylene oxide treatment, ethylene oxide treatment, gas plasma sterilization, gamma radiation or electron beam treatment, and peracetic acid (PAA) disinfection. Sterilization techniques which do not adversely affect the mechanical strength, structure, and biotropic properties of the submucosal tissue are preferred. For instance, strong gamma radiation may cause loss of strength of the sheets of submucosal tissue. Preferred sterilization techniques include exposing the graft to peracetic acid, 1-4 Mrads gamma irradiation (more preferably 1-2.5 Mrads of gamma irradiation) or gas plasma sterilization. Typically, the submucosal tissue is subjected to two or more sterilization processes. After the submucosal tissue is treated in an initial disinfection step, for example by treatment with peracetic acid, the tissue may be wrapped in a plastic

- 6 -

or foil wrap and sterilized again using electron beam or gamma irradiation sterilization techniques.

In accordance with one embodiment submucosal tissue is used as a tissue graft construct for the replacement or repair of damaged or diseased neurological related tissues. In particular the present submucosal tissue constructs have been found to promote the growth and proliferation of neurons. Accordingly, the present compositions can be used in a method of repairing damaged or diseased neurological related tissues in a warm-blooded vertebrate.

The submucosal tissue construct used in accordance with the present invention comprises intestinal submucosal tissue delaminated from both the tunica muscularis and at least the luminal portion of the tunica mucosa of warm-blooded vertebrate intestine, or a digest thereof. The construct can be combined with an added growth factor such as vascular endothelial growth factor, nerve growth factor or fibroblast growth factor or growth factor containing extracts of submucosal tissue. Alternatively, the tissue graft construct can comprise submucosal tissue in combination with peripheral neuronal tissue and optionally added growth factors.

In one embodiment, solid forms of submucosal tissue are combined with one or more growth factors by soaking the tissue in a buffered solution containing the growth factor. For example the submucosal tissue is soaked for 7-14 days at 4°C in a PBS buffered solution containing about 5 to about 500 mg/ml, or more preferably 25 to about 100 mg/ml of the growth factor. Submucosal tissue readily bonds to proteins and will retain an association with a bioactive agent for several days. However, to enhance the uptake of the growth factors into the submucosal tissue, the tissue can be partially dehydrated before contacting the growth factor solution. For compositions comprising fluidized, solubilized or guanidine extracts of submucosal tissue, lyophilized powder or solutions of growth factors can be directly mixed with the submucosal tissue. For example, fluidized or solubilized submucosal tissue can be mixed with a growth factor and then packed within a tube of submucosal tissue (or other biodegradable tissue). The open end of the tube is sealed shut after filling the tube with the fluidized or solubilized submucosal tissue.

- 7 -

In accordance with the present invention, submucosal tissue of a warm-blooded vertebrate is used to manufacture a tissue graft construct useful for inducing the repair of neurological tissue in a warm-blooded vertebrate. The manufacture comprises the steps of combining submucosal tissue of a warm-blooded vertebrate, or a digest thereof, with an added growth factor selected from the group consisting of vascular endothelial growth factor, nerve growth factor and fibroblast growth factor.

In one embodiment the submucosal tissue is used to manufacture a graft construct that directs the *in vivo* growth of neurons along a predetermined path. The manufacture comprises the steps of forming a tube of biodegradable material, and filling the tube with fluidized submucosal tissue. The tube should be formed to have a diameter about 0.5 mm to about 4 mm for peripheral nerve applications, and about 1 mm to about 2 cm for central nerve applications. In one embodiment the tube is formed from submucosal tissue wherein the submucosal tissue is manipulated to define a cylinder having a diameter of the preferred size. Typically the submucosal tissue is prepared directly from intestinal tissue and thus has a generally cylindrical shape. The tissue can be manipulated to define a cylinder having the preferred diameter by suturing or otherwise securing the graft longitudinally and removing the excess tissue. For example, the graft construct can be prepared by selecting a sterile glass rod having an outer diameter equal to the desired diameter of the lumen of the formed graft construct. The glass rod is introduced into the graft lumen, redundant tissues is then gathered, and the desired lumen diameter is achieved by suturing along the length of the graft or by using other art recognized tissue securing techniques.

Alternatively, a tube of submucosal tissue can be formed by wrapping strips of submucosal tissue onto a mandrel wherein the wrapped submucosal tissue is overlapped leaving no portion of the underlying mandrel exposed. See U.S. Provisional Application Serial No. 60/032,679, the disclosure of which is expressly incorporated herein. The submucosal tissue can be spirally wrapped onto a mandrel as a continuous piece of submucosal tissue, and multiple strips of submucosal tissue can be used to form the tubular constructs. The wrapped submucosal tissue is then compressed under dehydrating conditions and the tubular prosthesis is removed from the mandrel. The

- 8 -

amount of overlap in a spirally wrapped construct in accordance with this embodiment ranges between 10 to 60% of the width of the previous strip and more preferably the overlapped portion is a 50% overlap.

5 Upon formation of the biodegradable tube, the tube is filled with fluidized or solubilized submucosal tissue and the tube is sealed at one or both ends of the tube using art recognized methods (including clamping, suturing, binding pastes, and compression under dehydrating conditions). Alternatively the tube can be sealed at one or both ends of the tube before being filled with fluidized/solubilized tissue. The tube can then be filled by injecting fluidized/solubilized tissue into the lumen through the use of a
10 syringe.

The submucosal tissue graft constructs of the present invention are used to repair neurological related tissues and more particularly components of the central and peripheral nervous system. The method comprises contacting the site in need of repair with a composition comprising intestinal submucosal tissue delaminated from both the
15 tunica muscularis and at least the luminal portion of the tunica mucosa of warm-blooded vertebrate intestine. The submucosal tissue can be used, for example, in sheet, strip, braid or loop form and surgically implanted at the site in need of repair. The submucosal tissue composition can also be administered in a fluidized form and injected into the warm-blooded vertebrate at the site in need of repair. Finally the composition may comprise
20 fluidized submucosal tissue filled cylinder of submucosal tissue.

In one embodiment in accordance with the present invention, the submucosal tissue constructs are used to induce the formation of neurological related tissue between endogenous neurological tissue structures in a warm blooded vertebrate. The method comprises the steps of surgically implanting a tissue graft composition
25 comprising submucosal tissue of a warm-blooded vertebrate, into the host to bridge the endogenous neurological tissue structures and induce endogenous neurological related tissue growth between the bridged neurological structures.

When submucosal tissue comprising the tunica submucosa and basilar portions of the tunica mucosa including the lamina muscularis mucosa and the stratum
30 compactum, is used in non-fluidized form, it is preferred that it be implanted so that the

- 9 -

stratum compactum contacts the surface tissues most prone to forming adhesions with the graft material.

In accordance with one embodiment damage to the spinal cord can be repaired by manually separating adjacent longitudinal neural fibers in the spinal cord, wherein the separations or incisions run parallel to the axons of the neurons of the spinal column and penetrate through the dura mater, arachnoid and pia mater layers. Strips of submucosal tissue are surgically implanted into the vertical separations or in natural sulci and thus are in direct contact with neurological related tissues and are held in place by those tissues. Alternatively, sheets of submucosa tissue can be used to wrap the exterior of the damaged area to promote repair of the damaged tissues. Optionally sutures can be utilized to secure the submucosal tissue in its desired location.

In applications where the spinal cord has been transected, the submucosal tissue can be positioned between the two severed ends to bridge the gap and serve as a framework that directs the growth of neurons of the two severed ends towards one another. Fluidized forms of submucosa can also be used in accordance with the present invention to repair damaged or diseased neurological related tissues. Advantageously the fluidized forms can be injected into the site in need of repair and thus can be used in a less invasive procedure to induce the proliferation of endogenous neurological related tissues.

In accordance with one embodiment a tissue graft composition, comprising submucosal tissue of a warm-blooded vertebrate, is administered to a warm-blooded vertebrate at a site in need of endogenous neurological related tissue growth in an amount effective to induce endogenous neurological related tissue growth at the site the composition is administered. The biotropic properties of submucosal tissue promote the growth of neurological tissue along "tracts" as defined by the path of the implanted submucosal tissue. Accordingly the growth of neurons, including neurons of central and peripheral nervous system, can be directed to a site in need of innervation. In one embodiment the growth of neurological tissue is "directed" through the use of a tissue graft construct comprising a tube filled with fluidized submucosal tissue. In this embodiment healthy neuronal tissue is inserted into one end of the tube and is placed in direct contact with the fluidized submucosal tissue contained within the tube. The

- 10 -

opposite end of the tube is then placed at or near the site in need of innervation. The tube is fixed in place and provides an *in vivo* conduit for new neuronal growth and innervation of the desired site. The tube of fluidized submucosal tissue can also be utilized to repair transected nerves, wherein the two ends of the transected nerve are inserted into the tube to induce reattachment of the severed ends and restore nerve function.

Each of the following methods can be used in conjunction with the tissue graft constructs of the present invention to provide a conduit for directed *de novo* growth of neuronal tissues. In one embodiment submucosal tissue is prepared in the shape of a tube having a lumen and two open ends. In one embodiment, the tube of submucosal tissue is directly implanted into the host organism and the end of a damaged nerve fiber can be inserted into the lumen of the submucosal tissue tube. A syringe is then used to fill the tube with comminuted or solubilized submucosal tissue. Alternatively, submucosal tissue can be formed in the shape of a tube, filled with fluidized/solubilized submucosal tissue and sealed at each end. The sealed tube of submucosal tissue can then be stored until needed. In one embodiment the sealed tube of submucosal tissue is inserted into the host organism and fixed in place using techniques known to those skilled in the art. The inserted graft construct provides a conduit for new neuronal tissue growth. In one preferred embodiment, a slit or hole is cut into the tube of submucosal tissue and a damaged or resected end of a nerve tissue is inserted through the slit or hole and into the lumen of the tube.

The submucosal tissue used in accordance with the present invention can be used alone or combined with added growth factors such as vascular endothelial growth factor, nerve growth factor or acidic fibroblast growth factor. In addition peripheral nerve implants can be used in combination with submucosal tissue to enhance the repair of neuronal tissues. The term peripheral nerve implant as used herein refers to neuronal tissue harvested from the peripheral nervous system of a warm blooded vertebrate, and preferably autologous peripheral neuronal tissue. Additional components can be added to the neuronal tissue graft compositions that provide the compositions with structural support for applications involving the spinal cord, especially where portions of the spinal column are missing or need to be replaced. For example hydroxyapatite and/or other

- 11 -

biocompatible calcium containing minerals can be combined with the graft composition, or metal posts or wires can also be used in combination with the submucosal tissue to give additional structural support for the replacement tissue.

The submucosal tissue graft constructs of the present invention can also be
5 utilized to promote the growth and proliferation of other central nervous-associated and support tissues. Submucosal tissue enhances the repair of glial cells, and dura mater, arachnoid and pia mater tissues.

In one embodiment the submucosal tissue is used as a dural substitute, formed as a patch tissue graft shaped to cover a defect or hole formed in the endogenous
10 dura mater. Presently available options for dura mater substitute materials have significant limitations: autologous materials are frequently inadequate in quantity and are obtained with the associated morbidity of additional incisions, and the handling characteristics of synthetic sheets are poor compared to biological materials. In addition, concern has been raised regarding long term risks of hemorrhage from tissue reaction to
15 synthetic graft materials. Cadaveric dura is expensive, occasionally limited in supply, and has only fair handling characteristics. Of greater concern is its documented role as a vector in the transmission of the slow viruses such as Jakob-Creutzfeldt disease.

Submucosal tissue provides an excellent dural substitute since this material does not invoke an adverse immunologic response and induces proliferation of
20 endogenous cells which invade and ultimately replace the graft with endogenous cells. An experiment was conducted utilizing rat hosts to confirm the utility of submucosal tissue compositions as dural substitutes.

As described in Example 1, submucosal tissue implanted in a rat following dural resection functions as a suitable dural substitute. At 28 days after implantation
25 remodeling of the submucosal tissue has begun, as is indicated by the presence of spindle cells, aggressive neovascularization, and eosinophilic staining of the connective tissue matrix. The incorporation and remodeling of the graft takes place in the absence of any adverse effects on the underlying cerebral cortex.

30

Example 1**Submucosal Tissue as a Dural Substitute***Experimental Design and Surgical Procedure*

Twenty medium size Sprague-Dawley laboratory rats were anesthetized
5 (ketamine 90 mg/kg and xylazine 10 mg/kg, IM) and placed in a stereotaxic head frame to
stabilize the cranium. The scalp was shaved, prepped with chlorhexadine, and infiltrated
with 1% lidocaine. Following incision of the fascia at the superior temporal line, the
temporalis muscles were elevated laterally through a midline scalp incision, exposing the
parietal convexities. Bihemispheric parietal craniectomies, approximately 4 mm X 8 mm,
10 were made with an electric hand drill and burr. The dura, a thin, nearly transparent
membrane in the rat, was resected at the craniectomy sites under loop magnification.
Care was taken not to injure the underlying cerebral cortex.

Small intestinal submucosa graft material, in sheet form, was prepared in
accordance with the present invention and sterilized by exposure to 0.1% peracetic acid.
15 The graft was cut to the appropriate size and placed as an onlay graft over one convexity,
with the graft orientated so that the stratum compactum surface faced the cerebral cortex.
The contralateral hemisphere received no graft, thus serving as a control for host response
to the operative procedure. In two animals the bone fragment was replaced. The wound
was irrigated with normal saline and closed with staples. A single post operative dose of
20 ampicillin (25mg/kg, SQ) was given. Immediate post operative care included placement
on a heating pad, covering with a towel, turning every 15 minutes until awake and
moving, and monitoring heart rate and respirations. The animals were monitored daily for
the occurrence of seizures or neurological deficits, appetite and fluid intake, and weight.
Ten of the rats were sacrificed by barbiturate overdose (150 mg/kg, IC) 7 days after graft
25 placement. The remaining ten rats were sacrificed 28 days after graft placement.

Histological Preparation and Evaluation

Three of the twenty rats died of anesthetic related complications in the
early post operative period. The remaining seventeen rats recovered uneventfully from

- 13 -

the procedure without evidence of seizures, infection, or neurologic deficit. Eight rats were sacrificed at day 7. Nine were sacrificed at day 28.

Following sacrifice, the rats were perfused with formalin via carotid artery catheters. The cranium was then fixed in formalin and decalcified. Six micron thick sections were cut, stained with hematoxylin and cosin, and prepared for histologic examination. The tissues examined included the interfaces of the graft with the cortex, bone, and scalp. The control side was similarly prepared.

Microscopic evaluation of the specimens was augmented with quantification of the cellular infiltrate, vascularity, and thickness of the defect site using an image analysis system (Optimus Image Analysis System; Bioscan, Inc; Edmonds, WA). Data for the submucosal graft implant treated defect site were compared to the control site at both the 7 day and 28 day time points. The numerical scores given to the remodeling tissues were based upon the criteria given in Table 1. The values were compared using the Student's T test. The total score for the respective groups was used to test the null hypothesis that there was no difference between the morphologic changes seen in the submucosa filled versus the non-submucosa filled defect sites at either 7 or 28 days. A p-value less than 0.05 was considered significant.

Table 1. Quantitative Histological Assessment of Submucosa Dural Onlay Grafting vs. Control

20

25

30

Group	Thickness	Vascularity	Cellular Density	Total Score
7 day Graft (n = 8)	1.1 ± 0.2	0.9 ± 0.2	1.4 ± 0.2	3.4 ± 0.8 ^a
7 day Control (n = 8)	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.4 ± 0.1
28 day Graft (n = 9)	1.9 ± 0.3	1.9 ± 0.4	0.8 ± 0.3	4.6 ± 1.1 ^a
28 day Control (n = 9)	1.1 ± 0.3	0.8 ± 0.2	0.3 ± 0.2	2.2 ± 0.5

^a value is significantly different from the value in the control group at a p-value of < 0.05

- 14 -

The submucosa graft sites are compared with the control sites using thickness, vascularity, and cellular density as the scoring criteria. The values listed represent the mean value \pm S.E.M.

5		
	<u>Criteria</u>	<u>Score</u>
		<u>Description of Score</u>
	Thickness	
	0 =	< 100 μ M
10	1 =	100 - 200 μ M
	2 =	> 200 μ M
	Vascularity	
	0 =	0 - 1 vessel cross sections / 100, μ M ²
15	1 =	2 - 4 vessel cross sections / 100 μ M ²
	2 =	> 5 vessel cross sections / 100 μ M ²
	Cellular density	
	0 =	Total cell area: extracellular matrix material < 0.5
20	1 =	Total cell area: extracellular matrix material 0.5 - 1.0
	2 =	Total cell area: extracellular matrix material > 1.0
25		

Histologic evaluation showed graft infiltration by spindle shaped mononuclear cells, deposition of connective tissue, and neovascularity. Furthermore, histological analysis revealed distinct differences between the defects repaired with submucosa tissue versus the control (i.e. defects left to heal without any material placed at the defect site). The total score for the respective groups were compared using the Student's T test, significance being accepted for a p value < 0.05. A significant difference between the histologic scores of the submucosa graft site and control site was found at 7 days (3.4 ± 0.8 vs. 0.1 ± 0.1) and at 28 days (4.6 ± 1.1 vs. 2.2 ± 0.5). No evidence of adverse effect on the underlying cortex was observed.

At day 7, the main differences between the two groups were the cellular infiltrate, vascularity, and the thickness of the connective tissue deposited at the defect

- 15 -

site. These morphologic changes were compared in a semiquantitative fashion as defined in Table 1. This method of comparison showed increased thickness, increased vascularity, and greater cellular infiltration of the submucosa treated defects versus the non submucosa treated control defects. The mononuclear cells which were seen within and
5 around the submucosa material at day 7 often showed a spindle shape and were surrounded by eosinophilic staining extracellular matrix (ECM) material. The remodeling submucosa material showed a large number of capillary sized blood vessels in contrast to those observed in the non-submucosa control defects.

By 28 days, the cellular infiltrate had moderated in the submucosa-filled
10 defects and the amount of ECM had increased. The eosinophilic staining connective tissue in and around the submucosa showed orientation in the direction that would extend from one edge of the cut calvaria to the opposite edge. There was also moderate organization of the connective tissue seen in the non-submucosa defects; however the amount of material present was much less than the submucosa defect sites. The
15 submucosa material itself was not discernible by day 28. The ECM appeared homogeneous in these H&E stained sections. The cellular infiltrate was much less at day 28 than at day 7 and virtually all of the cells present were consistent with spindle shaped mesenchymal cells.

Occasional adhesions were noted between the ECM within the defect site
20 and the underlying cerebral cortex in both the submucosa and non-submucosa sides. None of the specimens showed changes consistent with encephalitis, degeneration, or necrosis.

EXAMPLE 2

25 Submucosal tissue as a dural substitute in the canine model.

Experimental Design and Surgical Procedure

Eight medium size mongrel dogs (20 - 30 kg) were anesthetized, intubated, and placed in the sternal supine position (induction with 2% thiopental 1.0 mg/kg, intravenous; maintenance with isoflurane 1% - 2%; atropine 0.5 mg/ml,
30 intravenous). Ophthalmic ointment was administered. The scalp was shaved, prepped

- 16 -

with chlorhexadine, and infiltrated with 1% lidocaine. Through a midline scalp incision and following incision of the fascia at the superior temporal line, the temporalis muscle was elevated laterally exposing the parietal convexity. A 2 X 3 cm temporoparietal craniotomy was made with an electric hand drill and burr. Bleeding bone edges were
5 waxed. The dura was resected at the craniotomy sites under loop magnification. Care was taken to avoid injury to the underlying cerebral cortex.

The submucosal tissue graft material was harvested and sterilized by exposure to 0.1% peracetic acid in 20% ethanol for 120 minutes. The material was cut to the appropriate size and placed with the compacted basal layers of the tunica mucosa
10 toward the cerebral cortex and secured with braided nylon suture. In five animals, a contralateral procedure was performed in which the resected autologous dura was used to close the defect, thus serving as a control for host response to the operative procedure.

In three animals, the contralateral side was subjected to dural resection with intestinal submucosal replacement sixty days after the initial procedure. The
15 craniotomy flap was replaced, the wound irrigated with normal saline, and closed with staples. Tribiotic ointment, sterile head dressing, and an Elizabethan collar were applied. Antibiotic treatment consisting of cephalexin 1000mg, PO, b.i.d., one day preoperatively and for three days postoperatively was administered. Immediate post operative care included covering with a blanket and monitoring heart rate and aspirations. Post
20 operative pain was treated with butorphanol (2.4 mg, intramuscular) and Ace Promazine (2.0 mg, intramuscular). The animals were monitored for the occurrence of seizures or neurological deficits, appetite and fluid intake, and weight. Sacrifice was by barbiturate overdose (150 mg/kg, intracardiac) on 7, 30, 60, 90, and 120 days after initial graft
placement and 7, 30, and 60 days following repeat intestinal submucosal tissue exposure.
25 Preceding sacrifice 5 cc of CSF was aspirated via suboccipital puncture and examined for different cell count.

Histological Preparation and Evaluation

Following sacrifice, the head and neck of the dogs were perfused with
30 formalin via carotid artery catheters. The cranium was then fixed in formalin and

- 17 -

decalcified. Following embedding in paraffin, six micron thick sections of the surgical sites were cut, stained with hematoxylin and eosin, and prepared for histologic examination. The tissues examined included the interfaces of the graft with the cerebral cortex, bone, and scalp.

5

Results

All eight dogs recovered uneventfully from the procedure without evidence of seizures, infection, or neurologic deficit. Repeat grafting was not accompanied by clinical evidence of sensitization to the submucosal tissue.

10 At day 7 following implantation, an intense mononuclear cell response into the intestinal submucosal tissue was present, with extensive neovascularization and deposition of disorganized extracellular matrix around the submucosal tissue. However, there was no evidence for involvement of the underlying cerebral cortex. There was a small number of spindle shaped cells consistent with fibroblasts around and within the
15 graft site.

 At every examination time following the 7 day time period (30, 60, 90, and 120 days) a well organized eosinophilic staining dense connective tissue was evident, with no evidence of the submucosal tissue remaining by day 60. No meningocerebral adhesions were seen. In the dogs subjected to a second exposure to intestinal submucosal
20 tissue, a response indistinguishable from those with single intestinal submucosal tissue exposure was found.

 In some animals, in both the test site and the control site, there was a mild inflammatory reaction of the pia mater adjacent to the cerebral. Since this was present in both the control site and the test site it likely represents a response to the surgical
25 procedure. In no animal was there evidence for involvement of the cerebral cortex itself. Neovascularization was more intense at the 7 and 30 day time points in the submucosal tissue sites compared to the control sites. There was no evidence for an immune mediated graft reaction of host sensitization in either the initial implants or in the reimplanted animals. There were no abnormalities in the serum chemistry or CSF cytology found in
30 any of the post operative samples.

- 18 -

Discussion

Current options for a dural substitute include autologous materials (e.g. pericranium, temporalis fascia and tensor fascia lata), lyophilized cadaveric materials (e.g. dura mater and tensor fascia lata), xenogeneic biomaterials (bovine and ovine pericardium) bovine dura, and reconstituted bovine collagen sponge) and synthetic materials (e.g. expanded polymer fluorethylene, Silastic sheets, Dacron sheets, Vicryl mesh). However, each of these materials is associated with significant limitations.

Autologous materials are frequently inadequate in quantity and are obtained with the associated morbidity of additional incisions. The handling characteristics of synthetic sheets are poor compared to biological materials. In addition, concern has been raised regarding long term risks of hemorrhage from tissue reaction adjacent to the graft. Cadaveric dura is expensive, occasionally limited in supply, and has only fair handling characteristics. Its documented role as a vector in the transmission of Jakob-Creutzfeldt disease severely limits its appeal. Likewise, recent evidence of transmission to humans of bovine spongiform encephalopathy raises concern over bovine based neurological tissues.

The dura mater consists primarily of Type I collagen, and thus collagen-based products are reasonable candidates for dural substitution. Furthermore, although relative biological inertness had previously been considered a desirable implant characteristic, there is increasing awareness of the potential advantages of an induced favorable biologic response to grafting. Indeed, fibroblasts and endothelial cells have been shown to invade along a reconstituted collagen scaffolding, replacing it by newly synthesized collagen. Several collagen based preparations have been investigated and appear to exhibit many of these characteristics. Preparations reported for dural substitution include processed porcine peritoneum and dermis, bovine corium and pericardium, ovine pericardium, and multiple reconstituted products of human placenta. Graft incorporation with subsequent resorption is reported for each of these materials. In addition, they produced neither acute immune reactions nor epileptogenic meningoencephalitic scars when applied to cortex.

- 19 -

Nevertheless, only bovine pericardium is commercially available. Thus, a non-bovine based collagen preparation with favorable biological properties appears to offer many benefits while avoiding many disadvantages. The acellular submucosal material of the present invention provides a novel material for dura mater substitute constructs. In addition to the absence of an adverse immunologic response, there is demonstrated a distinctive remodeling of the submucosal tissue and incorporation into the host tissue. The final form of the remodeled dural graft appears to be histologically indistinguishable from native tissue. In addition, exposure to the intestinal submucosal tissue two months following initial grafting was not accompanied by adverse clinical events. Routine CSF cytology provided no evidence for adverse host response to the submucosal tissue graft.

EXAMPLE 3

In vitro growth and differentiation of neuronal cells cultured on submucosal tissue.

15

Pheochromocytoma cells (PC12 cells) are neuronal cells which grow as spherical chromaffin cells in the absence of nerve growth factor (NGF) but differentiate to form sympathetic-like neurons upon exposure to nerve growth factor treatment. PC12 cells are an established and well studied cell line that has been utilized as a model system to study neuronal differentiation and proliferation. The response of PC12 cells to exposure to various forms of submucosa tissue (dehydrated, ETO, PAA) was investigated to determine if submucosal tissue would promote the differentiation, growth and proliferation of neuronal cells.

25 Cell Culture

Rat Pheochromocytoma (PC12) cells were obtained from American Type Culture Collection (Rockville, MD) and were cultured as described by Green Tishler (1983). Initially the cells were grown to confluence in monolayers attached to plastic 75 cm flasks. PC12 cells were maintained in RPMI 1640 medium supplemented with 10% horse serum, 5% fetal bovine serum, 1% L-glutamine and 100 units/ml penicillin and 100

30

- 20 -

ug/ml streptomycin. Cells were seeded in plastic 6 well culture dishes precoated with 0.2 mg/ml collagen. PC12 cells, serving as a positive control, were differentiated into sympathoblast-like cells by treatment with nerve growth factor (NGF) at a concentration 50 mg/ml for 7-10 days in RPMI. The medium containing NGF was changed every other day during this period. After 10 days in culture PC12 cells were terminally differentiated and dependent upon NGF for survival.

Chemicals

Horse serum and Bovine serum were purchased from Hyclone (Logan, UT). RPMI from Gibco BRL (Grand Island, NY). Small intestine submucosa was prepared in accordance with the procedures described in the present specification. All other chemicals were purchased from Sigma Chemical Company.

1. Growth and Differentiation Studies

To demonstrate PC12 cells differentiate on submucosa, cells were seeded (50,000/ml) in plastic uncoated 6 well culture plates, each well containing a 1-2 inches square of submucosa (dehydrated submucosa was preserved as sterile sheet, ETO, PAA) and RPMI media for 10 days. The submucosa was cut in small pieces and placed in cell culture plate wells by sterile forceps and the cells were pipetted onto the submucosa. Cells were observed under a inverted light microscope every day for the changes in PC12 cells.

Every 2, 4, 6, 8 and 10 days pictures were taken to compare the degree of differentiation of PC12 cells by observing quantitatively the number of neurites and neurite length. Each experiment was repeated three times (n=3) in triplicates. Differentiation of the cells was observed for cells in direct contact with the submucosa tissue as well as for cells that were not in direct contact with the submucosa tissue present in the well.

The following controls were used to compare the experimental observations, each control (comprising four wells) was repeated three times (n=3):

- 21 -

Positive Control: Cells were seeded in plastic 6 well culture plates precoated with 0.2 mg/ml collagen and in media containing 50 mg/ml NGF. Cells were grown for 10 days in RPMI containing NGF which was changed every two days.

5 Negative Control 1: Cells were seeded in plastic 6 well culture plates precoated with 0.2 mg/ml collagen in media containing no NGF.

Negative Control 2: In a second negative control cells were seeded in uncoated plastic 6 well culture plates in the media containing no NGF. Since the cells in negative control 2 started dying and floating after 2-3 days, this control was discarded and no experimental data was compared to this control.

10

Results:

PC12 neuronal cells cultured on submucosal tissue substrates in the absence of any added growth factors differentiate, proliferate and appeared to migrate on the substrate. A time-based study was conducted and observations were taken after 2, 4, 15 6, 8 and 10 days of culturing in order to correlate time of exposure to the submucosa substrate with changes in neuronal cell differentiation. PC12 cells differentiated on the submucosa substrate as early as one day after seeding. By the second day there was noticeable differentiation as compared to negative control. However, a greater number of cells differentiated and a greater degree of differentiation was observed in the cells after 20 two days of culturing. The qualitative differences between the controls and experimental cells continued after four days of culturing: by day four the positive control showed a greater degree of differentiation than the cells growing on submucosa and finally the negative control showed the least degree of differentiation.

After six days of culture, a visual comparison of the cells under the 25 microscope indicated there was an increase in number of cells. However many cells were floating and clumping. This result is very typical of PC12 *in vitro* cell cultures, and suggests that those cells that did not differentiate, continued to proliferate and as a result of higher number of cells they clumped. Cells cultured on submucosa by the sixth day had none to very small neurites as compared to positive control, and many cells at this stage 30 appear to migrate on the submucosa. A higher degree of differentiation was observed in

- 22 -

cells not in direct contact with the submucosa in the culture plate. That is, those PC12 cells immediately adjacent to, but not in direct contact with, the intestinal submucosal tissue showed good differentiation.

5 **Histological Studies:**

Time-based histological analysis was utilized to investigate when cells migration occurs on the submucosa substrate and whether or not the migrating cells remain differentiated or change to undifferentiated cells and lose neurites. Wells with submucosa material were flushed with PBS (2ml) and then subsequently fixed in saline
10 buffered formalin (4ml) overnight before they could be further processed for slide preparation. Samples in duplicates on days 2, 4, 6, 8 and 10 days of time based studies were provided for slide preparation. Slides are under preparation.

Experiments were also conducted to demonstrate which side of submucosa (mucosal or serosal) was able to better differentiate PC12 cells. Methods for seeding
15 PC12 cells on both sides of submucosa were similar as described above. It was observed that the mucosal side of submucosa appeared to be more venerable for differentiation of neuronal cells as compared to the other side of submucosa.

To observe the effect of Gamma irradiation on the differentiation of PC12 cells, PC12 cells were seeded on both sides of Gamma radiated submucosa. Preliminary
20 results indicate that the degree of differentiation of cells cultured on gamma radiated submucosa was higher than cells cultured on regular submucosa (hydrated ETO, PAA). This study was done only once (n=1) in triplicate, therefore repeated studies are required to confirm the results. However these initial results suggest the possibility of gamma radiation assisting in breaking/disturbing mechanical structure of submucosa and releasing
25 some sort of differentiating promoting factors to show increased differentiation.

To determine the effect of physically moving the culture plate ("Mechanical Disturbance Studies") on cell differentiation, PC12 cells were seeded on submucosa as described above. The seeded culture plate was not moved for 5 days and cells were monitored under the microscope. The experiment was conducted once (n=1)
30 in 6 wells. Physically moving the culture plate resulted in lower degree of differentiation,

- 23 -

implying that movement of the culture plate after seeding interferes with cell differentiation.

Two experiments were performed in duplicate to determine the effect of changing the media on cell differentiation. The RPMI media was replaced on 2, 4, 6, 8
5 and 10 days in the wells which consisted of cells seeded on submucosa mucosal side and submucosa abluminal side, negative control and control, where cells were seeded on uncoated plate without NGF. The experiment was repeated twice (n=2) with triplicates. Results from this study indicated that change in media had no significant effect in cell differentiation using either side of submucosa and negative control. However in the
10 control the cells did not float and die as early as the control cells cultured without a change in the media.

Example 4

Antibody Neutralization Studies of PC12 Cells cultured on Submucosal Tissue
15 Substrates

Materials & Methods

Cells: Rat Pheochromocytoma (PC12) cells were obtained from American Type Culture
20 Collection (Rockville, MD) and were cultured as described by Green Tishler (1983). Initially the cells were grown to confluence in monolayers attached to plastic 75 cm flasks. PC12 cells were maintained in RPMI 1640 medium supplemented with 10% horse serum, 5% fetal bovine serum, 1% L-glutamine and 100 units/ml penicillin and 100 ug/ml streptomycin. Passages 3-4 were used for these studies at a concentration of 40,000
25 cells/ml.

Culture plates: 12-well plates (Falcon) were treated overnight with 1 ml of a solution of 0.2 mg/ml rat tail collagen at 37°C. The collagen coated plates were used for the experiments within 3 days of coating.

30

- 24 -

Test Media: RPMI Complete media and Submucosal Tissue Conditioned Media (STCM) were used in these experiments. STCM was prepared by incubating serum-free RPMI 1640 stock media with PAA (0.1%) & gamma (1 MRad) treated submucosal tissue at a concentration of 2g submucosal tissue for each 15 ml of media. Treatment was for 5 48 hours at 37°C. Serum & other supplements were added to the submucosal tissue treated media to yield the STCM.

Growth Factors: Mouse 7S-NGF (BT-5023) was purchased from Harlan and was utilized at a concentration of 50 ng/ml. Recombinant bovine FGF2 (1363-719) was 10 purchased from Boehringer Mannheim and was used at a concentration of 10 ng/ml.

Antibodies: Anti-mouse NGF (1087-754) was purchased from Boehringer Mannheim and was used at a concentration of 150 ng/ml. Rabbit anti-bovine FGF2 (AB-33-NA) was purchased from R&D and used at a concentration of 40-50 µg/ml.

15
Method: PC 12 cells were plated in duplicate at a concentration of 40,000 cells/ml in 12-well plates containing 0.5 ml of RPMI medium or STCM. Growth factors and/or antibodies were added as appropriate. Cultures were evaluated for PC12 differentiation 48 hours after plating by counting 3-20X fields per well.

20
Differentiation: PC12 cells were considered differentiated if they exhibited at least one neurite-like extension which extended a minimum of 2 cell body diameters from the cell nucleus.

25 Results

RPMI Media Treatments: It was discovered that NGF & FGF2 both induced PC12 differentiation in the concentrations tested. Wells not containing growth factor did not differentiate. It was noted that the growth factor activity was effectively neutralized by 30 the addition of the respective neutralizing antibody to the neurite-inducing factor.

- 25 -

Addition of anti-NGF to the wells containing FGF2 also blocked differentiation in RPMI 1640 media, addition of anti-FGF2 to the wells containing NGF did not alter differentiation.

- 5 *STCM Treatments:* STCM induced differentiation of PC12 cells. Differentiation was effectively-blocked with the addition of anti-FGF2 neutralizing antibody. Addition of anti-NGF neutralizing antibody had no effect on differentiation.

Conclusions

10

1. STCM contains a substance that causes PC12 differentiation. The effect of this substance can be neutralized with the addition of anti-FGF2 neutralizing antibody.
2. PC12 differentiation in the STCM is not affected by the addition of anti-NGF
15 neutralizing antibody.
3. The anti-NGF neutralizing antibody may cross react with bovine FGF2. It is also possible that the antibody binds non-specifically to the FGF2 receptor.

20 **Example 5**

Sciatic Nerve Regeneration.

Experimental Design and Surgical Procedure

- Small intestinal submucosal graft material, in sheet form, was prepared in
25 accordance with the present invention and sterilized by exposure to 0.1% peracetic acid. A single sheet of submucosal tissue was formed in the shape of a tube having a luminal diameter of about 2 mm and the opposing ends of the sheet tissue were sutured longitudinally along the axis of the tube. The lumen of the submucosal tissue tube was filled with a suspension of comminuted submucosal tissue immediately before
30 implantation of the graft construct into the rat.

- 26 -

Eleven medium sized Sprague-Dawley laboratory rats were anesthetized (Ketamine 90 mg/kg and Xylazine 10 mg/kg, I.M.) and approximately 6 to 7 mm of the tibial branch of the sciatic nerve was resected in each of the rats. The resected portion was replaced with either an empty silastic tube (serving as the control) or a fluidized submucosal tissue filled tube construct. The two severed ends of the tibial branch were inserted into the prepared tubes, one severed nerve end being placed in the first end of the tube and the other severed nerve end inserted into the opposite ends of the tube such that a gap of about 6 to about 7 mm separated the two ends of the resected tibial branch. Five of the rats served as controls, having the severed nerve ends placed within an empty silastic tube, and six rats had the severed nerve ends inserted into submucosal tissue tubes in direct contact with the fluidized submucosal tissue (See Table 2). The graft constructs were fixed in place by suturing to the surrounding tissue. The wound was irrigated with normal saline and closed with staples. Immediately post-operative care included placement on a heating pad, covering with a towel, turning every 15 minutes until awake and moving, and monitoring heart rate and respirations.

The animals were monitored daily, and at 30 days all rats were observed using their legs only to push off. No grasping was recorded. Four animals were sacrificed on August 23, 1997. The two rats having the silastic tube implanted showed some signs of neuronal tissue growth and some directed growth along the tubes axis. However, in the submucosal tissue tube constructs, the resected ends of the tibial branch appeared to have reattached.

- 27 -

Table 2.
Experimental timetable

5	Group	Animal	Submucosal Tissue Tube Constructs	Silastic Tube	Post Dates
	30 Days	#1	7-23-97		8-23-97
		#2	7-24-97		
		#3		7-24-97	
		#4		7-25-97	
	60 Days	#5	7-28-97 died 8-4-97		9-20-97
		#6	7-28-97		
		#7		7-25-97	
		#8		7-28-97	
	120 Days	#9	7-29-97		11-17-97
		#10	7-29-97		
		#11		7-29-97	

10

- 28 -

Claims:

1. A tissue graft construct for promoting the repair of damaged or diseased neurological related tissues in a warm-blooded vertebrate, said construct comprising intestinal submucosal tissue delaminated from both the tunica muscularis and
5 at least the luminal portion of the tunica mucosa of warm-blooded vertebrate intestine, or a digest thereof and an added growth factor.
2. The construct of claim 1, wherein the growth factor is selected from the group consisting of nerve growth factor and fibroblast growth factor.
3. The tissue graft construct of claim 1 wherein the submucosal tissue
10 comprises a solid tube of submucosal tissue and fluidized submucosal tissue located in the lumen of the tube of submucosal tissue, wherein the fluidized submucosal tissue is selected from the group consisting of comminuted and solubilized submucosal tissue or extracted fraction thereof.
4. A tissue graft construct for repairing neurological tissues, said
15 composition comprising a biodegradable tube having a luminal diameter sized to receive nerve tissue; and
fluidized submucosal tissue selected from the group consisting of comminuted and solubilized submucosal tissue, wherein the fluidized submucosal tissue is located in the lumen of the tube.
- 20 5. The tissue graft construct of claim 4 wherein the biodegradable tube comprises submucosal tissue.
6. The tissue graft construct of claim 4 wherein the biodegradable tube has a luminal diameter of about 0.5 mm to about 2 cm.
7. A biodegradable dura mater substitute material comprising
25 intestinal submucosal tissue delaminated from both the tunica muscularis and at least the luminal portion of the tunica mucosa of warm-blooded vertebrate intestine, or a digest thereof, and formed as a patch tissue graft shaped to cover a defect or hole in endogenous dura mater.

- 29 -

8. The dural mater substitute material of claim 7 wherein the edges of the patch tissue graft are folded back onto themselves to provide reinforced regions for attachment to endogenous tissues.

9. The use of submucosal tissue of a warm-blooded vertebrate in the manufacture of a tissue graft construct useful for inducing the repair of neurological tissue in a warm-blooded vertebrate, said manufacture comprising the steps of combining submucosal tissue of a warm-blooded vertebrate, or a digest thereof, with an added growth factor selected from the group consisting of nerve growth factor and fibroblast growth factor.

10. The use of submucosal tissue of a warm-blooded vertebrate in the manufacture of a tissue graft construct useful for inducing the repair of neurological tissue in a warm-blooded vertebrate, said manufacture comprising the steps of forming a tube of submucosal tissue having a luminal diameter of about 0.5 mm to about 2 cm and filling the luminal of said submucosal tissue tube with fluidized submucosal tissue selected from the group consisting of comminuted and solubilized submucosal tissue.

11. The use of claim 10 wherein the ends of the tube of submucosal tissue are sealed shut.

12. A method for promoting the repair of damaged or diseased neurological related tissues in a warm-blooded vertebrate, said method comprising the step of contacting the site in need of repair with a composition comprising intestinal submucosal tissue delaminated from both the tunica muscularis and at least the luminal portion of the tunica mucosa of warm-blooded vertebrate intestine.

13. The method of claim 12 wherein the neurological tissue is dura mater.

14. The method of claim 12 wherein the neurological tissue comprises neurons of the central nervous system.

15. The method of claim 12 wherein said submucosal tissue is fluidized.

16. The method of claim 15 wherein said submucosal tissue is injected at the site in need of repair.

- 30 -

17. The method of claim 12 wherein said submucosal tissue is surgically implanted at the site in need of repair.

18. The method of claim 12 wherein said submucosal tissue is in powder form.

5 19. A method for inducing the formation of neurological related tissue between endogenous neurological tissue structures in a warm blooded vertebrate at a site in need of endogenous neurological related tissue growth, said method comprising surgically implanting a graft composition comprising submucosal tissue of a warm-blooded vertebrate to bridge the endogenous neurological tissue structures to induce
10 endogenous neurological related tissue growth between the bridged neurological structures.

20. The method of claim 19, wherein the graft composition is fluidized and is administered by injection into the warm-blooded vertebrate.

21. The method of claim 19, wherein the graft composition is
15 administered by surgically implanting the composition into the warm-blooded vertebrate.

22. The method of claim 19, wherein the neurological related tissue is dura mater.

23. The method of claim 19 wherein the neurological related tissue comprises neurons of the central nervous system.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/16294

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 35/37, 35/38

US CL : 424/550, 551

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/550, 551

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONEElectronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	US 5,275,826 A (BADYLAK ET AL.) 04 January 1994, see column 1, lines 40-45 and column 2, lines 23-27.	1-11 ----- 1-23
X ----- Y	US 4,902,508 A (BADYLAK ET AL.) 20 February 1990, see column 2, lines 42-57.	1-11 ----- 1-23
A	US 5,360,610 A (TICE ET AL.) 01 November 1994 see entire patent, especially column 2.	1-23

☐

Further documents are listed in the continuation of Box C.

☐

See patent family annex.

* *A* *B* *L* *O* *P*	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance earlier document published on or after the international filing date document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	*T* *X* *Y* *A*	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art document member of the same patent family
--------------------------------------	---	--------------------------	--

Date of the actual completion of the international search

06 NOVEMBER 1997

Date of mailing of the international search report

23 DEC 1997

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

DWAYNE C. JONES

Telephone No. (703) 308-1235

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/16294

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

REGISTRY, CA, CAPLUS, BIOTECH, WPIDS, USPATFUL, BIOSIS, MEDLINE, EMBASE search terms: nerve growth factor, fibroblast growth factor, (nerve or fibroblast)(w)(growth factor or growth factor) and (tissue graft or graft), (submucos? or tissue or mucos?) and (luminal or lumen)